

CM What is claimed is:

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B1 1. An isolated mannanase which is

(a) a polypeptide encodable by the mannanase enzyme encoding  
5 part of the DNA sequence cloned into the plasmid present in  
Escherichia coli DSM 12197, or

(b) a polypeptide comprising an amino acid sequence as shown in  
positions 31-330 of SEQ ID NO:2, or

(c) a polypeptide encodable by the DNA sequence as shown in  
10 positions 91-990 or positions 91-1470 of SEQ ID NO:1, or

(d) an analogue of the polypeptide defined in (a) or (b) which  
is at least 65% homologous with said polypeptide, or a fragment  
of (a), (b) or (c).

15 2. The mannanase according to claim 1 which is derivable from a  
strain of *Bacillus* sp.

3. The mannanase according to claim 2 which has

i) a relative mannanase activity of at least 60% in the pH range  
20 7.5-10, measured at 40°C;

ii) a molecular weight of  $34 \pm 10$  kDa, as determined by SDS-PAGE;  
and/or

iii) the N-terminal sequence ANSGFYVSGTTLYDANG.

25 4. An isolated polynucleotide molecule comprising a DNA sequence  
encoding an enzyme exhibiting mannanase activity, which DNA  
sequence comprises:

(a) the mannanase encoding part of the DNA sequence cloned into  
the plasmid present in *Escherichia coli* DSM 12197;

30 (b) the DNA sequence shown in positions 91-1470 in SEQ ID NO 1,  
preferably position 91-990, or its complementary strand;

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(c) an analogue of the DNA sequence defined in (a) or (b) which is at least 65% homologous with said DNA sequence;

(d) a DNA sequence which hybridizes with a double-stranded DNA probe comprising the sequence shown in positions 91-990 in SEQ ID NO 1 at low stringency;

(e) a DNA sequence which, because of the degeneracy of the genetic code, does not hybridize with the sequences of (b) or (d), but which codes for a polypeptide having exactly the same amino acid sequence as the polypeptide encoded by any of these DNA sequences; or

a DNA sequence which is a fragment of the DNA sequences specified in (a), (b), (c), (d), or (e).

5. The cloned DNA sequence according to claim 4, in which the DNA sequence encoding an enzyme exhibiting mannanase activity is obtained from a microorganism.

6. An isolated polynucleotide molecule encoding a polypeptide having mannanase activity which polynucleotide molecule hybridizes to a denatured double-stranded DNA probe under medium stringency conditions, wherein the probe is selected from the group consisting of DNA probes comprising the sequence shown in positions 91-990 of SEQ ID NO:1, the sequence shown in positions 91-1470 of SEQ ID NO:1 and DNA probes comprising a subsequence of positions 91-990 of SEQ ID NO:1 having a length of at least about 100 base pairs.

7. An expression vector comprising the following operably linked elements: a transcription promoter; a DNA segment selected from the group consisting of (a) polynucleotide molecules encoding a polypeptide having mannanase activity comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 91 to nucleo-

tide 990, (b) polynucleotide molecules encoding a polypeptide having mannanase activity that is at least 65% identical to the amino acid sequence of SEQ ID NO:2 from amino acid residue 31 to amino acid residue 330, and (c) degenerate nucleotide sequences of (a) or (b); and a transcription terminator.

8. A cultured cell into which has been introduced an expression vector according to claim 7, wherein said cell expresses the polypeptide encoded by the DNA segment.

9. An isolated polypeptide having mannanase activity selected from the group consisting of:

(a) polypeptide molecules comprising an amino acid sequence as shown in SEQ ID NO: 2 from residue 31 to residue 330; and

(b) polypeptide molecules that are at least 65% identical to the amino acids of SEQ ID NO: 2 from amino acid residue 31 to amino acid residue 330.

10. The polypeptide according to claim 9 which is produced by *Bacillus sp.* I633.

11. An enzyme preparation comprising a purified polypeptide according to claim 9.

12. A method of producing a polypeptide having mannanase activity comprising culturing a cell into which has been introduced an expression vector according to claim 7, whereby said cell expresses a polypeptide encoded by the DNA segment; and recovering the polypeptide.

13. The preparation according to claim 11 which further comprises one or more enzymes selected from the group consisting of

proteases, cellulases (endoglucanases),  $\beta$ -glucanases, hemicellulases, lipases, peroxidases, laccases,  $\alpha$ -amylases, glucoamylases, cutinases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, pectin lyases, other mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases; and mixtures thereof.

10 14. An isolated enzyme having mannanase activity, in which the enzyme is (i) free from homologous impurities, and (ii) produced by the method according to claim 12.

15 15. A method for improving the properties of cellulosic or synthetic fibres, yarn, woven or non-woven fabric in which method the fibres, yarn or fabric is treated with an effective amount of the preparation according to claim 11 or an effective amount of the enzyme according to claim 1.

20 16. The method according to claim 15, wherein the enzyme preparation or the enzyme is used in a desizing process step.

25 17. A method for degradation or modification of plant material in which method the plant material is treated with an effective amount of the preparation according to claim 11 or an effective amount of the enzyme according to claim 1.

30 18. The method according to claim 17 wherein the plant material is recycled waste paper; mechanical, chemical, semichemical, kraft or other paper-making pulps; fibres subjected to a wetting process; or guar gum or locust bean gum containing material.

19. A method for processing liquid coffee extract, in which method the coffee extract is treated with an effective amount of the preparation according to claim 11 or an effective amount of the enzyme according to claim 1.

20. A cleaning composition comprising the enzyme preparation according to claim 11 or the enzyme according to claim 1.

21. The cleaning composition according to claim 20 which further comprises an enzyme selected from cellulases, proteases, lipases, amylases, pectin degrading enzymes and xyloglucanases; and a conventional detergent ingredient.

22. The cleaning composition according to claim 20 wherein the enzyme or enzyme preparation is present at a level of from 0.0001% to 2% pure enzyme by weight of total composition.

23. The cleaning composition according to claim 21 wherein the enzyme is present at a level of from 0.0001% to 2% pure enzyme by weight of total composition.

24. The cleaning composition according to claim 21 wherein the enzyme is an amylase.

25. The cleaning composition according to claim 24 which further comprises yet another enzyme selected from cellulase, protease, lipase, pectin degrading enzyme and xyloglucanase.

26. The cleaning composition according to claim 21 which comprises a surfactant selected from anionic, nonionic, cationic surfactant, and/or mixtures thereof.

27. The cleaning composition according to claim 21 which comprises a bleaching agent.

5 28. The cleaning composition according to claim 21 which comprises a builder.

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29. A fabric softening composition according to claim 21 which comprises a cationic surfactant comprising two long chain  
10 lengths.

30. A process for machine treatment of fabrics which process comprises treating fabric during a washing cycle of a machine washing process with a washing solution containing the enzyme  
15 preparation according to claim 11 or the enzyme according to claim 1.

31. An isolated mannanase which is  
(a1) a polypeptide encoded by the mannanase enzyme encoding part  
20 of the DNA sequence cloned into the plasmid present in Escherichia coli DSM 12180, or  
(b1) a polypeptide comprising an amino acid sequence as shown in positions 32-344 of SEQ ID NO:6, or  
(c1) an analogue of the polypeptide defined in (a) or (b) which  
25 is at least 85% homologous with said polypeptide, or a fragment of (a1), (b1) or (c1);  
(a2) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in Escherichia coli DSM 12433, or  
30 (b2) a polypeptide comprising an amino acid sequence as shown in positions 32-362 of SEQ ID NO:10, or

(c2) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a2), (b2) or (c2);

(a3) a polypeptide encoded by the mannanase enzyme encoding part  
5 of the DNA sequence cloned into the plasmid present in *Escherichia coli* DSM 12441, or

(b3) a polypeptide comprising an amino acid sequence as shown in positions 33-331 of SEQ ID NO:12, or

(c3) an analogue of the polypeptide defined in (a) or (b) which  
10 is at least 85% homologous with said polypeptide, or a fragment of (a3), (b3) or (c3);

(a4) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in *Escherichia coli* DSM 9984, or

15 (b4) a polypeptide comprising an amino acid sequence as shown in positions 166-488 of SEQ ID NO:14, or

(c4) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a4), (b4) or (c4);

20 (a5) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in *Escherichia coli* DSM 12432, or

(b5) a polypeptide comprising an amino acid sequence as shown in positions 68-369 of SEQ ID NO:16, or

25 (c5) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a5), (b5) or (c5);

(a6) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in  
30 *Escherichia coli* DSM 12849, or

(b6) a polypeptide comprising an amino acid sequence as shown in positions 29-320 of SEQ ID NO:22, or

(c6) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a6), (b6) or (c6);

(a7) a polypeptide encoded by the mannanase enzyme encoding part  
5 of the DNA sequence cloned into the plasmid present in Escherichia coli DSM 12180, or

(b7) a polypeptide comprising an amino acid sequence as shown in positions 301-625 of SEQ ID NO:26, or

(c7) an analogue of the polypeptide defined in (a) or (b) which  
10 is at least 85% homologous with said polypeptide, or a fragment of (a7), (b7) or (c7);

(a8) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in Escherichia coli DSM 12851, or

15 (b8) a polypeptide comprising an amino acid sequence as shown in positions 166-496 of SEQ ID NO:28, or

(c8) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a8), (b8) or (c8);

20 (a9) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in Escherichia coli DSM 12852, or

(b9) a polypeptide comprising an amino acid sequence as shown in positions 26-361 of SEQ ID NO:30, or

25 (c9) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a9), (b9) or (c9);

(a10) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in  
30 Escherichia coli DSM 12436, or

(b10) a polypeptide comprising an amino acid sequence as shown in positions 593-903 of SEQ ID NO:32, or



(c10) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a10), (b10) or (c10).

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